Effects of insulin and atrial natriuretic peptide on renal tubular sodium handling in sickle cell disease

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Ter Maaten, Jan C., Erik H. Serné, Wim Status van Eps, Pieter M. ter Wee, Ab J. M. Donker, and Rijk O. B. Gans. Effects of insulin and atrial natriuretic peptide on renal tubular sodium handling in sickle cell disease. Am. J. Physiol. Renal Physiol. 278: F499–F505, 2000.—We assessed the effect of insulin and atrial natriuretic peptide (ANP) on renal sodium handling in eight patients with sickle cell disease (SCD), who are characterized by loss of vasa recta and long loops of Henle, and matched control subjects. During insulin infusion (50 mU·kg⁻¹·h⁻¹), fractional sodium excretion decreased by 0.44 ± 0.72% (P = 0.13) in patients with SCD and by 0.57 ± 0.34% (P = 0.002) in control subjects, whereas fractional distal sodium reabsorption increased by 4.1 ± 1.5% (P < 0.001) and 3.0 ± 1.5% (P < 0.001), respectively. Low-dose (0.3 pmol·kg⁻¹·h⁻¹) ANP infusion did not affect renal sodium handling in patients with SCD but increased fractional sodium excretion by 0.34 ± 0.22% (P = 0.003) in control subjects. High-dose (2 µg/min) ANP increased natriuresis to a similar extent in both groups. Insulin's antinatriuretic effects predominated over the natriuretic effects of low-dose, but not high-dose, ANP. These data suggest that insulin's antinatriuretic effect is localized at a distal tubular site other than the long loops of Henle and that the long loops are involved in the natriuretic effect of low-dose ANP, possibly mediated by changes in medullary blood flow.

THE MOST PROMINENT RENAL EFFECT of exogenously administered insulin is sodium retention. Insulin exerts this antinatriuretic effect by increasing distal tubular sodium reabsorption, whereas, at the same time, proximal tubular sodium reabsorption decreases (9, 27, 29). It is not yet clear, however, whether insulin increases distal tubular sodium reabsorption along the loops of Henle or a more distal tubular site.

As opposed to insulin, the predominant renal effect of exogenous atrial natriuretic peptide (ANP) is stimulation of sodium excretion. The natriuretic effect of high doses of ANP appears to be multifactorial: a rise in glomerular filtration rate (GFR) and a reduction in both proximal and distal tubular sodium reabsorption (5). Rat studies have suggested that the natriuretic effect of low-dose ANP can be explained by an increase in the medullary blood flow through the vasa recta and, thus, an increase in renal interstitial pressure (21).

Given the opposite effects of insulin and ANP on renal sodium handling, the interaction or balance between both hormones may be important for (long-term) blood pressure regulation. In a study in normal subjects, the natriuretic effect of a low dose of ANP, itself without renal hemodynamic effects, abolished the antinatriuretic effect of insulin in the distal tubule (17). In contrast, insulin did not counteract the natriuretic effect of pharmacological dosages of ANP (30).

A unique human pathophysiological “model” to study the tubular localization of hormonal action is available in sickle cell disease (SCD). The hallmark of sickle cell nephropathy is obliteration of the vasa recta by sickling of red cells and subsequent loss of the long loops of Henle (4, 25). This specific renal defect in SCD offers the opportunity to discriminate between the long loops of Henle and more distal tubular sites by concomitant examination of patients with SCD and control subjects.

In the present study we have assessed the tubular localization of the effects of insulin and two doses of ANP on renal sodium handling, using the lithium clearance technique, in patients with SCD and normal control subjects. We also assessed the interaction of insulin and ANP regarding renal sodium handling by concomitant infusion of both hormones in each group of subjects.

METHODS

Subjects

Eight patients with SCD participated in the studies. Five of them had sickle cell anemia, two were heterozygous for hemoglobin S and C, and one was heterozygous for hemoglobin S and β-thalassemia. All had an impaired concentrating capacity of urine after 24-h thirsting the maximal urine osmolality amounted to 435 ± 51 (SD) mosmol/kgH₂O. The healthy volunteers were matched to the patients for age, gender, race and body mass index (Table 1). Blood pressure levels did not differ between both groups. The healthy volunteers had a normal hemoglobin A pattern as determined by hemoglobin electrophoresis.

All patients with SCD and healthy volunteers had a normal 75-g oral glucose tolerance test according to World Health Organization criteria. The patients did not use medications other than folic acid supplementation. Informed consent was obtained from all subjects. The protocol had been
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Study Design

Each subject underwent four studies in randomized order on 4 separate days, at least 7 days apart. The week before each study, the subjects adhered to a 150-mmol sodium diet, and compliance was ascertained by measuring the 24-h urinary sodium excretion the last 2 days before the studies. Lithium carbonate (300 mg) was taken at 10 PM on the evening before each study. After an overnight fast, all subjects came to the clinic at 8 AM. They were given 10 ml of sodium perchlorate each hour, reduced by the volume of glucose infusion to standard formulas. Fractional clearances were preferred to absolute clearances, because they correct for changes in GFR and sodium handling.

Study a: Insulin infusion. The hyperinsulinemic euglycemic clamp technique was used to assess sensitivity to insulin-mediated glucose uptake, as described previously (11, 29). In short, insulin (Velosulin; Novo Nordisk, Bagsvaerd, Denmark) was diluted to 40 ml with 45 ml of 0.9% saline and 5 ml of 20% human albumin, was infused in a primed, continuous manner at a rate of 50 µmol·kg⁻¹·h⁻¹ for 4 h. Normoglycemia was maintained by adjusting the rate of a 20% D-glucose infusion during the clamp clamp. Plasma insulin concentrations were measured by radioimmunoassay (immunoradiometric assay, Incstar, Stillwater, MN). Blood samples for measurement of sodium, lithium, and ANP were drawn halfway through the clamp. Plasma insulin concentrations were measured by radioimmunoassay (immunoradiometric assay, Medgenix Diagnostics, Fleury, Belgium).

Study b: ANP infusion. A low-dose ANP (Clinalfa, Läufelfingen, Switzerland) infusion of 0.3 pmol·kg⁻¹·h⁻¹, known to increase plasma ANP levels approximately twofold (18), was given for 2 h, succeeded by a high-dose ANP infusion of 2 µg/min for 2 h.

Study c: Insulin and ANP infusion. The combined infusions of insulin and ANP were given using the protocols of studies a and b.

Study d: Time-control experiment. Control experiments were carried out in a fashion identical to infusion of the same amount of solvent and with blood sampling at the same time intervals. Control experiments enabled us to correct for any circadian variation in the variables under evaluation.

To ensure adequate diuresis, 300 ml of water were given orally each hour, reduced by the volume of glucose infusion during the euglycemic clamp studies. Blood pressure and heart rate were measured by using a semicontinuous blood pressure-measuring device (Nippon Colin BP 103 N Sphygmonanometer, Hayashi, Komaki-City, Japan). Six measurements were performed during each 1-h clearance period.

Measurement of Renal Hemodynamics and Sodium Handling

Renal plasma flow (RPF) and GFR were measured by using the simultaneous infusion of [131I]hippuran and [125I]iothalamate, as described previously (1, 6). Briefly, a continuous infusion containing 100 µCi [131I]hippuran and 50 µCi [125I]iothalamate (Amersham, UK) in 100 ml of saline was administered at a rate of 12 ml/h, after a priming dose of 20 µCi [131I]hippuran and 10 µCi [125I]iothalamate had been given. Urine was collected by spontaneous voiding, and blood samples were drawn at the start and end of each clearance period. The activities of [131I]hippuran and [125I]iothalamate in plasma, urine, and diluted infusion solution (1:100) were determined in duplicate by using a well-type scintillation counter (1282 CompuGamma, Wallac). Calculations of the clearance rates were made by using the formulas 1 · V/P (plasma clearance) and U · V/P (renal clearance), where V is volume of the infusion or urine in milliliter per minute, and P is cpm per milliliter of diluted infusion solution, U is cpm per milliliter urine, V is volume of the infusion or urine in milliliter per minute, and P is cpm per milliliter of plasma. RPF was calculated as the plasma clearance of [131I]hippuran (Chip). GFR was calculated as the renal clearance of [125I]iothalamate corrected for incomplete urine collection with the equation Cio (V/V/P) / Chip(U · V/P). Day-to-day coefficients of variation are 5.0 and 2.2%, respectively (6).

Sodium and lithium clearances were calculated according to standard formulas. Fractional clearances were preferred to absolute clearances, because they correct for changes in GFR as well as for dead space or incomplete voiding. Fractional proximal sodium reabsorption was calculated as (1 – CNa/Ci) · 100%, and fractional distal tubular sodium reabsorption as (1 – CNa/Cf) · 100%, where Ci, and CNa are lithium and sodium, respectively.

Laboratory Investigations

Blood samples for measurement of sodium, lithium, and ANP were drawn halfway through the clearance periods. Serum and urinary concentrations of lithium were measured by atomic absorption (atomic absorption spectrophotometer, Perkin Elmer, Norwalk, CT). Plasma ANP was determined by radioimmunoassay by using sheep antiserum (125I RIA Kit, Incstar, Stillwater, MN). Blood samples for measurement of plasma insulin were drawn four times during the second and fourth hour of the clamp. Plasma insulin concentrations were measured by radioimmunoassay (immunoradiometric assay, Medgenix Diagnostics, Fleury, Belgium).

Statistical Analysis

Between-group differences were analyzed with the unpaired Student's t-test. All variables were analyzed by ANOVA for repeated measurements to detect differences over time and between the studies, followed by paired t-tests. To avoid bias caused by circadian variation of the variables under evaluation, day-to-day coefficients of variation were 5.0 and 2.2%, respectively (6).
evaluation, the measurements during the control experiments were subtracted from the corresponding measurements obtained during each intervention before statistical analysis was performed (10). A value of \( P < 0.05 \) was considered to be significant. Data are expressed as means ± SD unless stated otherwise.

RESULTS

The 24-h urinary sodium excretion before the studies and the average baseline values of systemic and renal hemodynamics are provided in Table 1. Apart from hemoglobin and hematocrit, the baseline values of the four studies did not differ within both subgroups. Renal plasma flow tended to be higher in the patients with SCD than in the control subjects (\( P = 0.06 \)), but renal blood flow was similar.

Effects of Insulin Infusion

During the euglycemic clamp, glucose levels averaged 4.2 ± 0.3 mmol/l in patients with SCD and 4.2 ± 0.1 mmol/l in control subjects. Insulin-mediated glucose uptake (M value, mg kg\(^{-1}\)·min\(^{-1}\)) did not differ between patients with SCD and control subjects (7.5 ± 4.6 and 9.3 ± 2.8, respectively, \( P = 0.4 \)). The coefficient of variation of the blood glucose level during insulin infusion was 7.4 ± 2.2 and 9.4 ± 2.7%, respectively. Measurement of plasma insulin in patients with SCD was hampered by chronic low-grade hemolysis. In the normal subjects, plasma insulin levels amounted to 513 ± 103 and 521 ± 100 pmol/l during the second and fourth hour of the clamp, respectively.

Systolic and diastolic blood pressure did not change during insulin infusion in either group. RPF and GFR showed nonsignificant increases in patients with SCD, but RPF increased from 499 ± 66 to 590 ± 86 ml/min (\( P = 0.004 \)) and GFR from 111 ± 21 to 117 ± 22 ml/min (\( P = 0.017 \)) in the normal subjects.

The changes in fractional sodium excretion and segmental tubular sodium reabsorption are given in Tables 2 and 3. Changes in renal sodium handling were not different between patients with SCD and normal subjects. Compared with the time-control experiment, fractional sodium excretion decreased to a similar extent in both groups, by 0.44 ± 0.72% (\( P = 0.13 \)) in patients with SCD and by 0.57 ± 0.34% (\( P = 0.002 \)) in the control subjects (Fig. 1). Estimated fractional distal sodium reabsorption increased by 4.1 ± 1.5% (\( P < 0.001 \)) and 3.0 ± 1.5% (\( P < 0.001 \)), respectively (Fig. 1). At the same time, estimated fractional proximal sodium reabsorption during insulin infusion decreased in both groups.

Effects of ANP Infusion

Measurement of plasma ANP levels in patients with SCD was hampered by chronic low-grade hemolysis. In the normal subjects, plasma ANP levels amounted to 35 ± 13, 46 ± 31, and 991 ± 759 pg/ml at baseline and during the second and fourth hour of the ANP infusion, respectively.

During low-dose ANP infusion, systemic and renal hemodynamics did not change in both groups. During high-dose ANP infusion in patients with SCD, mean arterial blood pressure (MAP) and RPF did not change, but GFR increased from 114 ± 36 to 130 ± 36 ml/min (\( P = 0.006 \)). In control subjects, MAP decreased from 88 ± 11 to 82 ± 11 mmHg (\( P = NS \)), RPF decreased from 504 ± 84 to 429 ± 72 ml/min (\( P = 0.017 \)), and GFR increased from 114 ± 20 to 123 ± 20 ml/min (\( P = 0.006 \)).

The low-dose ANP infusion did not affect renal sodium handling in patients with SCD (Table 2). In the control subjects, low-dose ANP increased fractional sodium excretion by 0.34 ± 0.22% (\( P = 0.003 \)), accompa-

Table 2. Effects of insulin and atrial natriuretic peptide on fractional sodium excretion and distal and proximal tubular sodium reabsorption in patients with sickle cell disease

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>2h</th>
<th>P2</th>
<th>4h</th>
<th>P4</th>
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<tbody>
<tr>
<td>C(_{\text{Na}}/\text{GFR}, %)</td>
<td></td>
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</tr>
<tr>
<td>Insulin</td>
<td>1.54 ± 0.93</td>
<td>1.43 ± 0.64</td>
<td>NS</td>
<td>1.23 ± 0.69</td>
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<tr>
<td>ANP</td>
<td>1.28 ± 0.90</td>
<td>1.39 ± 1.04</td>
<td>NS</td>
<td>5.00 ± 3.36</td>
<td>0.005</td>
</tr>
<tr>
<td>ANP + insulin</td>
<td>1.27 ± 0.43</td>
<td>1.39 ± 0.71</td>
<td>NS</td>
<td>4.37 ± 2.49</td>
<td>0.007</td>
</tr>
<tr>
<td>Timecontrol</td>
<td>1.29 ± 0.32</td>
<td>1.44 ± 0.79</td>
<td>NS</td>
<td>1.42 ± 0.86</td>
<td>NS</td>
</tr>
<tr>
<td>1−(C(_{\text{Na}}/\text{GFR}), %)</td>
<td></td>
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<tr>
<td>Insulin</td>
<td>92.6 ± 4.2</td>
<td>94.8 ± 2.9</td>
<td>0.004</td>
<td>95.9 ± 2.5</td>
<td>0.001</td>
</tr>
<tr>
<td>ANP</td>
<td>94.0 ± 1.9</td>
<td>94.1 ± 2.9</td>
<td>NS</td>
<td>84.3 ± 6.5</td>
<td>0.003</td>
</tr>
<tr>
<td>ANP + insulin</td>
<td>93.7 ± 3.9</td>
<td>94.2 ± 3.5</td>
<td>0.04</td>
<td>87.4 ± 6.0</td>
<td>0.013</td>
</tr>
<tr>
<td>Timecontrol</td>
<td>93.3 ± 2.3</td>
<td>94.2 ± 3.2</td>
<td>NS</td>
<td>92.5 ± 3.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD. Significant differences during each study over baseline values: *\( P < 0.05 \), †\( P < 0.01 \), and ‡\( P < 0.001 \).

Table 3. Effects of insulin and atrial natriuretic peptide on fractional sodium excretion and distal and proximal tubular sodium reabsorption in normal subjects

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
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<th>P2</th>
<th>4h</th>
<th>P4</th>
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<tr>
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<td></td>
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</tr>
<tr>
<td>Insulin</td>
<td>1.70 ± 0.58</td>
<td>1.24 ± 0.45</td>
<td>0.019</td>
<td>1.29 ± 0.70</td>
<td>0.002</td>
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<tr>
<td>ANP</td>
<td>1.16 ± 0.42</td>
<td>1.53 ± 0.60</td>
<td>0.003</td>
<td>4.65 ± 3.01</td>
<td>0.01</td>
</tr>
<tr>
<td>ANP + insulin</td>
<td>1.32 ± 0.50</td>
<td>1.08 ± 0.37</td>
<td>NS</td>
<td>4.47 ± 1.82</td>
<td>0.001</td>
</tr>
<tr>
<td>Timecontrol</td>
<td>1.66 ± 0.54</td>
<td>1.69 ± 0.66</td>
<td>NS</td>
<td>1.82 ± 0.70</td>
<td>NS</td>
</tr>
<tr>
<td>1−(C(_{\text{Na}}/\text{GFR}), %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Insulin</td>
<td>92.9 ± 2.1</td>
<td>95.3 ± 1.4</td>
<td>0.014</td>
<td>95.6 ± 1.7</td>
<td>0.0008</td>
</tr>
<tr>
<td>ANP</td>
<td>94.5 ± 1.2</td>
<td>92.4 ± 3.8</td>
<td>NS</td>
<td>86.0 ± 6.1</td>
<td>0.008</td>
</tr>
<tr>
<td>ANP + insulin</td>
<td>94.5 ± 1.7</td>
<td>95.9 ± 1.1</td>
<td>0.004</td>
<td>87.8 ± 3.3</td>
<td>0.0008</td>
</tr>
<tr>
<td>Timecontrol</td>
<td>93.2 ± 1.8</td>
<td>93.0 ± 2.2</td>
<td>NS</td>
<td>92.9 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>1−(C(_{\text{Na}}/\text{GFR}), %)</td>
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<td></td>
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</tr>
<tr>
<td>Insulin</td>
<td>76.2 ± 4.9</td>
<td>74.2 ± 5.1</td>
<td>0.008</td>
<td>71.9 ± 6.1</td>
<td>0.003</td>
</tr>
<tr>
<td>ANP</td>
<td>78.0 ± 3.7</td>
<td>79.0 ± 3.6</td>
<td>NS</td>
<td>71.1 ± 8.4</td>
<td>0.04</td>
</tr>
<tr>
<td>ANP + insulin</td>
<td>76.1 ± 3.9</td>
<td>73.8 ± 3.1</td>
<td>NS</td>
<td>64.5 ± 5.8</td>
<td>0.0000</td>
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<tr>
<td>Timecontrol</td>
<td>75.6 ± 3.6</td>
<td>76.3 ± 4.4</td>
<td>NS</td>
<td>74.8 ± 4.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD. Significant differences during each study over baseline values: *\( P < 0.05 \), †\( P < 0.01 \), and ‡\( P < 0.001 \).
baseline and during the second and fourth hour, respectively.

In patients with SCD, MAP and RPF did not change, but GFR gradually increased from 108 ± 34 to 113 ± 31 ml/min (P = NS) during low-dose ANP and to 125 ± 34 ml/min (P = 0.008) during high-dose ANP. In control subjects, MAP gradually decreased from 90 ± 8 to 86 ± 9 mmHg (P = NS) during low-dose ANP, and to 79 ± 11 mmHg during high-dose ANP (P = 0.012). RPF increased from 504 ± 94 to 572 ± 127 ml/min (P = 0.002) during low-dose ANP and subsequently dropped to 485 ± 71 ml/min during high-dose ANP. GFR increased from 111 ± 19 to 119 ± 25 ml/min (P = 0.044), and to 124 ± 22 ml/min (P = 0.0006), respectively.

During the combined infusion of insulin and ANP in the control subjects, fractional sodium excretion decreased by 0.25 ± 0.33% (P = not significant vs.
baseline, P < 0.001 vs. ANP; Fig. 2). Changes in fractional distal and proximal sodium reabsorption during infusion of insulin and low-dose ANP were also similar to the changes during infusion of insulin only (Fig. 2).

Insulin did not attenuate the natriuretic effects of high-dose ANP in either patients with SCD or in the control subjects.

**DISCUSSION**

By using the unique human pathophysiological model of sickle cell nephropathy, the major observations of the present study are 1) a similar effect of insulin on renal sodium handling in patients with SCD and matched controls, 2) a natriuretic effect of low-dose ANP in normal subjects but not in patients with SCD, and 3) an overriding of the antinatriuretic effects of insulin over the natriuretic effects of low-dose ANP in healthy subjects.

SCD is characterized by specific renal abnormalities. Loss of long loops of Henle is obviously the most conspicuous renal defects in SCD (25). This specific abnormality enabled us to study the tubular site of action of insulin and ANP along the human nephron. Nevertheless, several reservations have to be made with regard to the interpretation of our results. First, the patients with SCD were a heterogenous group. However, it has previously been shown that loss of long loops of Henle is present in both patients with a homozygous and those with a double heterozygous sickle cell disorder (26), as illustrated by the impaired urinary concentrating capacity in all study subjects. Second, because hematocrit was reduced in SCD, we cannot exclude that rheological properties of blood could have affected vascular responses to ANP and insulin. Third, apart from loss of long loops of Henle, additional distal tubular function defects can be present in SCD (4). Fourth, the lithium clearance technique gives an estimate and not a quantitative measurement of segmental tubular sodium handling (23). To improve the accuracy of the method, we applied appropriate precautions such as performing the experiments in the sodium-replete state (23). The accuracy of the lithium clearance technique is not known in SCD. However, the observation that our patients with SCD tended to have higher proximal tubular sodium reabsorption than the controls by using this technique is in agreement with an enhanced proximal tubular function in SCD as measured by the clearances of β₂-microglobulin and phosphate (4). Fifth, the similar renal blood flow and GFR between patients with SCD and normal subjects possibly reflect some nephron loss in our middle-aged group of sickle cell patients, because young subjects with SCD are characterized by increases in RPF, renal blood flow, and GFR, whereas renal blood flow and GFR decrease with ageing (4, 14).

Insulin increased fractional distal tubular sodium reabsorption significantly in both groups, in line with most previous in vivo studies (9, 27, 29). The similar effect of insulin on distal tubular sodium reabsorption in patients with SCD and normal subjects suggests that this antinatriuretic effect of insulin is not localized in the long loops of Henle. It is unclear at which other distal tubular site insulin's antinatriuretic effect is localized. A role of the thick ascending limb of Henle's loop of the cortical nephrons has been suggested by micropuncture studies in hyperinsulinemic euglycemic rats (16). This suggestion is supported by the observation that insulin-binding capacity is highest along the thick ascending limb of Henle's loop in the rabbit nephron (19). In vitro studies of isolated rat nephron segments have shown that insulin stimulated Na⁺-K⁺-ATPase-mediated rubidium uptake in the cortical and medullary collecting tubules (8).

The observation that low-dose ANP induced natriuresis by reducing distal tubular sodium reabsorption in normal subjects but not in patients with SCD supports the notion that low-dose ANP inhibits sodium reabsorption in the long loops of Henle by increasing medullary blood flow and renal interstitial pressure (21). The presence of such an inhibitory action of ANP on sodium transport gains support from rat studies that showed that the natriuretic response to ANP was abolished after chemical papillectomy and after removal of the renal capsule (12, 13). It is not likely that ANP exerts its natriuretic effect by decreasing medullary hypertonicity (2). Studies in the isolated perfused rabbit loop argue against a direct inhibitory action of ANP on sodium transport (17).

Alternatively, low-dose ANP could inhibit sodium reabsorption in the collecting ducts (5). The inner medullary collecting duct appears to be the most important tubular site of action of ANP (5, 24, 28). Although no data are available from the literature, it is likely that the inner medullary collecting duct is damaged in SCD. ANP could act both directly and indirectly, again, via an increase in medullary blood flow and interstitial pressure to decrease sodium reabsorption.

High-dose ANP induced natriuresis by an increase in GFR and a decrease in proximal and distal tubular sodium reabsorption, as previously recognized (5). The results of our study show that these effects of high-dose ANP are similar in normal subjects and patients with SCD. This could suggest that the long loops of Henle do not play a relevant role in mediating the natriuretic effects of high-dose, in contrast to low-dose, ANP. Unfortunately, comparison of the natriuretic effects between both groups is hampered by a decline in blood pressure and RPF in the normal subjects, but not in the patients with SCD. It has previously been shown that the distal tubular action of ANP in humans is pressure dependent (7).

The effects of the combined infusions of insulin and ANP on renal sodium handling are especially interesting in the normal subjects because low-dose ANP only induced natriuresis in these subjects. In view of the supposedly different tubular points of action of insulin
and low-dose ANP, as discussed above, the final effect on renal sodium excretion will be determined by the balance of the action of both hormones. The results of our study show that, regarding the doses given, the effects of insulin predominate over the effects of low-dose ANP on renal sodium handling, whereas insulin does not attenuate the natriuretic effects of high-dose ANP. Of note, the degree of hyperinsulinaemia obtained during insulin infusion was in the higher physiological range (20), whereas plasma ANP concentrations were in the more average physiological and pharmacological range during low- and high-dose ANP infusion, respectively (5).

The different outcome of our study with regard to a previous study of Miller et al. (18), who found that low-dose ANP counteracted the sodium-retaining effect of insulin, may be due to larger increases in ANP concentrations in Miller’s study, despite similar ANP infusion rates. It has previously been recognized that similar ANP infusions may result in marked individual differences in plasma ANP levels (5).

To summarize, the results of our study suggest that insulin and low-dose ANP affect renal sodium handling in different parts of the distal nephron. Low-dose ANP exerts its natriuretic effect probably in the earlier part of the distal nephron, i.e., along the long loops of Henle, whereas insulin exerts an antinatriuretic effect in later segments of the distal nephron. In addition, insulin’s antinatriuretic effect is able to compensate for an increased sodium delivery in the more distal nephron during concomitant ANP infusion in normal subjects. It can be speculated that an impaired balance between both hormonal actions may contribute to abnormal sodium retention and blood pressure elevation in the long term.

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